

B² It is another object of the present invention to provide a host cell which expresses large amounts of INGAP [protein].

Replace the paragraph beginning column 1, line 47 with the following:

B³ It is an object of the present invention to provide a recombinant construct for expression of biologically active INGAP [protein].

Replace the paragraph beginning column 1, line 50 with the following:

B⁴ Another object of the invention is to provide a method for isolating INGAP [protein] from a recombinant host cell.

Replace the paragraph beginning column 1, line 52 with the following:

B⁵ These and other objects of the invention are achieved by providing the art with a recombinant construct for expression of biologically active INGAP [protein] comprising:

Replace the paragraph beginning column 2, line 4 with the following:

B⁶ In yet another embodiment of the invention a host cell is provided. The host cell comprises a recombinant construct comprising a first nucleotide sequence encoding amino acid[s] residues 27 to 175 of SEQ ID NO: 6 operably linked to a transcriptional initiation site and a translational initiation site, wherein a second nucleotide sequence encoding a signal peptide is not present immediately 5' of said first nucleotide sequence. These and other embodiments of the invention which will be apparent to those of skill in the art provide a practical source of INGAP [protein] in amounts suitable for use in preclinical and clinical situations.

Replace the paragraph beginning column 3, line 8 with the following:

B 7 The coding sequence of amino acid residues 27-175 of INGAP [protein] are included in the constructs. Preferably the entire signal sequence is deleted. However, it is possible that only a portion of the signal sequence must be deleted to obtain excellent expression. Thus some portion of the signal sequence might be retained in the constructs.

Replace the paragraph beginning column 9, line 39 with the following:

B 8 In order to test for the overexpression of [the] INGAP [protein], discontinuous denaturing polyacrylamide gel electrophoresis was performed on the dialyzed protein solution using the Hoefer SE250 Might Small II apparatus. The separating gel was prepared with 15% acrylamide, 1.35% bis-acrylamide in 375 mM Tris buffer at pH 8.8 with 0.05% sodium dodecyl sulfate. Polymerization was induced by addition of 0.05% ammonium persulfate and 20 μ l TEMED/15 ml solution. The solution was placed in the gel plate apparatus for polymerization. The stacking gel was poured with the same solution, except the Tris buffer was 125 mM at pH 6.8, and the acrylamide concentration was 4%. The protein samples were diluted 1:1 with sample buffer (125 mM Tris-Cl, pH 6.8, 4% SDS, 20% glycerol, and 10% 2-mercaptoethanol).

Replace the paragraph beginning column 10, line 19 with the following:

B 9 The gel showed a protein band of approximately 19 kD that was prominent in the bacterial lysate from transfected cells and in the elution fraction from pH 4.5 on nickel/NTA (FIG.1). This protein was not represented in any of the other samples. This is consistent with the size of INGAP [protein] and with interaction of the inserted histidine tagging region with the nickel/NTA column matrix.

Replace the paragraph beginning column 10, line 61 with the following:

B 10
ECL of the blot revealed strong protein recognition of the overexpressed 19 kD proteins in the whole lysate from transfected bacteria (IBL) and the pH 4.5 fraction that were visualized on the SDS-PAGE gels (FIG. 2). In addition, there was a protein band recognized in both bacterial lysates at 40 kD, implying that this protein is weakly recognized and is a bacterial protein rather than a product of the transfection. Finally, there was a light band at 14 kD recognized by the antibody in both the transfected bacterial lysate and in the pH 4.5 fraction. This may either be another protein or a lytic fraction of [the] INGAP [protein]. Given the engineering done to produce [the] INGAP [protein] it is most likely a lytic fraction of INGAP.

Replace the paragraph beginning column 11, line 7 with the following:

B 11
In summary, we have been able to express INGAP [protein] in a prokaryotic system by excluding the 5' UTR and the signal peptide and insertion of the new construct into a new vector. The resultant protein is of the predicted molecular size of INGAP monomer and reacts with the antibody to INGAP in a Western analysis. The protein shares with INGAP peptide the ability to induce ductal cell proliferation.

Replace the paragraph beginning column 15, line 31 with the following:

B 12
This example describes denaturing metal affinity protein chromatography isolation of his tagged-INGAP [protein] without signal peptide. (Procedure for 2 cell pellets from five 150 mm plates each of pEBVHis-INGMAT transfected COS-7 cells).

Replace the paragraph beginning column 16, line 45 with the following:

B 13
In summary, we have been able to express INGAP [protein] in an eukaryotic system by excluding the 5'UTR and the signal peptide. The resultant protein is of the predicted molecular